Bull. Org. mond. Santé
Bull. Wld Hlth Org. } 1969, **41**, 643-645

Future Influenza Vaccines and the Use of Genetic Recombinants *

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Genetic recombination of influenza viruses provides the possibility of immediate reassortment and combination of genes and gene products in a single step. Thus, genetic variants with desirable attributes for vaccine production can be produced by deliberate genetic manipulation of viruses rather than by the empirical " hit or miss " methods of the past. Recombination of a high-yield laboratory strain (A0/PR/8) with a low-yield Hong Kong virus (Aichi strain) produced a high-yield recombinant virus (X-31) of Hong Kong antigenicity suitable for vaccine production. It is proposed that a prefabricated " library " of recombinants might anticipate the mutations which may arise in the future and also that live virus vaccines of greater stability may be produced by recombination of new and old viruses.

It is important to appreciate that influenza vaccines of the past and present are, in fact, empirically selected genetic viral variants with desirable properties; i.e., optimal growth characteristics, " attenuation " of virulence, thermal stability and requisite antigenicity. Selection of these variants from uncloned viral stocks of variable history has usually been hit or miss and unsystematic by such empirical methods as, for example, mouse-lung passage to increase viral yield in chick embryos.

Obviously, it is more sensible to attempt a correlation of identifiable genetic attributes (markers) of the virus with desirable vaccine properties. Then one can proceed to manipulate the virus genetically to select optimal clones: (1) by screening of multiple viral clones, (2) by chemically induced mutation and/or selection through environmental pressures (e.g., *ts* mutants), or (3) by genetic recombination of viruses.

Recombination is analogous to sexual reproduction in its potential for reassortment and recombination of genes and gene products. Thus, the deliberate mating of 2 or more viruses, each bearing a desired trait, can be effected and an appropriate progeny virus can be selected without need for tedious " adaptation " until appropriate mutants, if any, become manifest. It is predictable that recombination, with its reassortment of genes, will lessen the probability of expression of any polygenic characteristic, such as virulence which is dependent on combinations of genes. Therefore, a kind of " instant attenuation " can be provided by recombination of a wild type with established laboratory strains of lesser virulence and a virus of the required antigenicity can then be selected out [e.g., A*x* Vir1 Vir2 Vir3 (virulent) × A2 Avir1 Avir2 Avir3 → A*x* Avir1 Vir2 Avir3 (avirulent)]. However, new phenotypes can arise with recombination so that the possibility of increased virulence cannot be excluded. For example, a recombinant of A0/NWS and A2/RI/5$^+$ viruses, X-7, acquired a capacity to elute from erythrocytes that was not characteristic of either parental virus (Laver & Kilbourne, 1966). Recombination of genes had conferred on this hybrid the highly active neuraminidase of the A2 strain together with the relatively weak red-cell binding capacity of A0/NWS virus, so that its phenotype with respect to red-cell interaction was a new pattern of enhanced dissociation or elution from the cells. However, in this instance, the

* This investigation was conducted in part under the auspices of the Commission on Influenza, Armed Forces Epidemiological Board, and was supported in part by the US Army Medical Research and Development Command, Department of the Army, under Research Contract No. DA-17-69-C-9137; in part by US Public Health Service Research Grant AI-09304 from the National Institutes of Allergy and Infectious Diseases; in part by the Health Research Council of the City of New York under Contract No. U-1023.

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virulence of X-7 in any of 3 test systems was intermediate between that of its parents.

IMPLICATIONS OF RECOMBINATION FOR PRODUCTION OF LIVE VIRUS VACCINES

If, as seems likely, live virus vaccines assume more importance in the control of influenza, then the vexing problem of maintaining optimal infectiousness for man may be met by the provision of chick-embryo-adaptive genes from laboratory strains of virus in concert with those wild-type viral genes coding for the desired new antigen. Thus, the apparently inevitable de-adaptation to man coincident to adaptation of new viruses to an alien host (the chick embryo) might be circumvented by lessening the wild-type strain's requirement for an evolutionary flux of mutants as it strives to survive in the new host.

RAPID PRODUCTION OF HIGH-YIELDING VACCINE VIRUS

The control of influenza depends upon the availability of vaccine. Paradoxically (and predictably), at those times when vaccine is most needed (i.e., when new mutants appear) the new viruses available for vaccine production, because of their limited passage in the egg, are usually not suitable. It should be appreciated that not only is a 4-fold–8-fold difference in titre reflected by a proportionately greater egg requirement and hence manufacturing expense, but it may determine whether or not the small manufacturer can risk undertaking vaccine production at all. Therefore, any method that aids in the enhancement of influenza virus yields is potentially important.

The principle was clearly established 10 years ago that recombination of a low-yielding (A2) strain with a standard laboratory strain (A0/PR/8) could lead to the rapid emergence of recombinant virus with the desired (A2) antigenicity and the growth characteristics and haemagglutination titre of the older adapted strain (Kilbourne & Murphy, 1960). These experiments were conducted 2 years after the major A2 mutation of 1957, by which time higher-titre vaccine strains had become available, so that the results of these studies were not useful at that time.

When a strain of Hong Kong virus first became available to our laboratory on 20 September 1968 we immediately embarked on a similar experiment, again using A0/PR/8 as the growth-conferring parent. In less than 2 weeks a Hong Kong-like virus of increased titre had been isolated and within a month it had been recloned, checked for antigenicity and genetic stability and made available for distribution. Although low yields of virus still plagued vaccine manufacturers at that time, no great enthusiasm greeted the appearance of the recombinant, which exceeded the titre of standard Aichi strains by 4-fold–6-fold. At least one reason for this fact was the history of monkey kidney passage of the particular Hong Kong strain which had been employed as a parent, but the lateness of the hour (it was then mid-October) also dampened the interest of most manufacturers, some of whom had almost completed production.

Because it seemed important to reaffirm the principle of 1959, and to apply the genetic tool of hybridization for the first time to artificial immunization, recombination of a Hong Kong strain of unimpeachable pedigree (the Aichi vaccine strain provided by the Division of Biologics Standards) was again carried out, again with A0/PR/8 as the other parent. Recombination was effected by simultaneous inoculation of chick embryos with heat-inactivated Aichi and infective A0/PR/8. Subsequent selection depended upon (1) suppression of the parental or recombinant virus of A0 serotype with specific antiserum and (2) limiting diluting passage to permit the emergence of Hong Kong-like virus of good growth potential. This interaction and selection can be symbolized as: HKg × A0G → HKg + A0G + HKG + A0g, if G indicates good growth capacity and g the reverse. This hypothetical virus mixture + A0 antiserum → HKg + HKG; the latter can be expected to emerge at high dilution passage with screening dependent upon viral yield (i.e., HA titre). Again, a high-yielding recombinant, X-31, was obtained. In the accompanying table the properties of this virus are compared with the viruses from which it was derived. The yield of haemagglutinating virus is similar to the A0/PR/8 while antigenic analysis by any of 4 different techniques identified the envelope protein as identical with Hong Kong virus. Of particular note is the confirmation of earlier studies (Kilbourne & Murphy, 1960) that enhanced growth capacity was associated with predominantly spherical viral morphology. Thus, a convenient biophysical marker may identify the traits associated with high-yielding virus.

The virus X-31 is now in pilot commercial production and by the time this report has been published it will have been tested for antigenicity in

PROPERTIES OF X-31 AND PARENTAL VIRUSES

Virus	Antigens [a]		Yield in eggs (HA titre)	Optimal growth at 35°C	Viral morphology	Elution from erythrocytes [b]
	Haemagglutinin	Neuraminidase				
PR/8	PR/8	PR/8	8 192	+	Spherical	+
HK [c]	HK	HK	256	−	Filamentous	−
X-31	HK	HK	8 192	+	Spherical	+

[a] Antigenic characterization defined by haemagglutination inhibition, neuraminidase inhibition, plaque inhibition and plaque-size reduction.

[b] First noted by Dr Geoffrey Schild.

[c] HK = Division of Biologics Standards 5th-egg-passage Aichi strain—no monkey kidney passage.

man in volunteer challenge experiments and in field trials.

Other potential applications of recombination to vaccine production include: (1) Establishment of a "library" of prefabricated recombinants in an attempt to anticipate recombination in nature of antigens presently known and which may lead to the evolution of new variants. For example, combination of the haemagglutinin of A/Equi-2/63 (cross-reactive with the haemagglutinin of Hong Kong virus) and the neuraminidase of a recent A2 strain (e.g., A2/Texas/68) identical to the HK enzyme (Schulman & Kilbourne, 1969) can be expected to yield a virus very like Hong Kong in antigenicity. We are at present trying to produce such a recombinant as an exercise in "retrospective anticipation". (2) The strains stored in the "library" should include pedigreed high-yield parental viruses ready for recombination with new strains (human or animal), as they evolve, to effect their rapid "adaptation" to the vaccine-producing host system. (3) Repeated recombination may reveal new antigens; as envelope polypeptides coded by the reassorted genes are put together in differing contexts, new avidity, reactivity or even new antigenicity may emerge. The markedly changed reactivity of the A0 neuraminidase in combination with the haemagglutinin of A/Equi-1 virus illustrates this point (Kilbourne, 1968).

Whether or not recombination is the mechanism of evolution of new strains in nature, the same trick can be used in the laboratory to the detriment of influenza and the benefit of man.

ACKNOWLEDGEMENTS

The able technical assistance of Barbara Pokorny and Jan Meisels is gratefully acknowledged.

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